

PHOTOPERIODIC TERMINATION OF DIAPAUSE IN AN INSECT¹

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Since the work of Kogure (1933) on the effect of photoperiod on diapause in the commercial silkworm, diapause in many insect species has been found to be photoperiodically induced (see reviews by Lees, 1955; de Wilde, 1962). The European corn borer, *Ostrinia nubilalis*, displays a photoperiodically induced facultative diapause in the final (fifth) larval instar (Mutchmor and Beckel, 1958, 1959; Beck and Hanec, 1960).

Diapause is defined as a state of arrested development in which the arrest is enforced by a physiological mechanism (Beck and Hanec, 1960). Diapause is, therefore, distinguishable from quiescence or dormancy that is enforced by unfavorable environmental conditions. Under natural conditions, diapause is eventually terminated and morphogenesis resumed. The physiological processes involved in the termination of diapause constitute developmental changes on a biochemical level, and have been termed "diapause development" by Andrewartha (1952). Andrewartha defined diapause development as (1952, p. 53) "the physiological development, or physiogenesis, which goes on during the diapause stage in preparation for the active resumption of morphogenesis." It is, therefore, the process of reversing (or replacing) the physiological mechanism enforcing the diapause state.

Experimental work on diapause development has dealt mainly with the low-temperature treatments necessary to terminate diapause; the reviews of Andrewartha (1952) and Lees (1955) discuss many examples of diapause development in eggs, larvae, pupae, and adults. In a few instances, termination of diapause has been found to be photoperiodically induced without a previous exposure of the insects to low temperatures (Baker, 1935; Paris and Jenner, 1959; Shakhbazov, 1961). According to the definitions employed above, photoperiodic termination of diapause must also involve diapause development.

The intensity of diapause, as measured by the length of time required to complete diapause development, varies widely among the species studied; a few days are required for *Loxostege sticticalis* (Pepper, 1937), but several months are needed in the case of *Melanoplus bivittatus* (Church and Salt, 1952). The intensity of diapause may also vary among individuals of the same species, depending on how long they have been exposed to diapause-inducing conditions. De Wilde *et al.* (1959) reported that diapause in the Colorado potato beetle, *Leptinotarsa decemlineata* Say, was photoperiodically reversible shortly after the adults displayed diapause behavior, but not a few days later. Hogan (1962) found that embryonic

¹ Approved for publication by the director of the Wisconsin Agricultural Experiment Station. This study was supported in part by a research grant (RG-7557) from the National Institutes of Health.

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diapause in the cricket *Acheta commodus* was more intense after 14 days of incubation at 23° C. than after only 7 days at that temperature.

Babcock (1924) reported that larval diapause in the European corn borer could be terminated only after the larvae had been subjected to about 6 weeks of freezing or near-freezing temperatures. This finding has been generally accepted, and in the absence of any photoperiodic treatment of the diapause larvae, is verifiable. In a recent study, Beck and Apple (1961) reported that diapause could be revoked in laboratory-reared borers by subjecting them to long-day photoperiods shortly after they had entered the diapause state. A low-temperature exposure was not required for such diapause termination, and the authors expressed doubt as to whether or not the larvae had been fully in diapause. They referred to such easily broken diapause as an "incipient" rather than "true" diapause.

The study here presented was undertaken in an effort to determine whether or not diapause in the European corn borer can vary in intensity under different conditions, and also to elucidate the relationship between photoperiod and the completion of diapause development.

MATERIAL AND METHODS

The European corn borers used in this study were from a restricted natural population occurring near Madison, Wisconsin. The use of a defined population was necessary because of the demonstration of significant differences in photoperiodic responses among different geographical populations of this species (Beck and Apple, 1961). Overwintering borers were collected from the field in the fall of the year, and were stored at 5° C. As needed, groups of stored borers were incubated at 30° C. for pupation and emergence. The progeny of these insects were used in the experiments described below, except where field borers are indicated. The times of collection and storage conditions for field borers are indicated in the appropriate sections.

The laboratory borers were reared aseptically on purified diets according to the rearing techniques described by Beck and Smissman (1960). All experiments were run at 30° C. with the exception of those treatments involving a temperature cycle or storage at 5° C.

The experiments were carried out in B.O.D. constant temperature incubators that had been modified to incorporate a thermistor temperature control system (Thermistemp Temperature Control Model 71, Yellow Springs Instrument Company, Yellow Springs, Ohio). Control of photoperiod was effected through the use of 7-day cycle programmers wired to two 14-watt fluorescent lights installed in the incubator. In experiments involving temperature changes, two methods were used. Symmetrical temperature cycles were obtained by using a clock motor to drive the thermistor temperature control unit through a prescribed cycle. The temperature reached a maximum of 31° C. and 12 hours later reached a minimum of 21° C. A temperature cycle with abrupt changes was obtained by having the thermistor temperature controller switched from 32.5° C. to 12° C. by a 24-hour programmer. This apparatus was set to give a temperature cycle with 16 hours at 32.5° C. and 8 hours at 12° C. The change from the maximum temperature to the minimum temperature took 1½ hours, while the change from the minimum to

the maximum temperature took one hour. The performance of the temperature-controlling apparatus was verified by a recording thermograph.

Throughout this paper the term *short-day* refers to a photoperiod consisting of 13 hours of photophase and 11 hours of scotophase, and the term *long-day* refers to a photoperiod with a 16½-hour photophase and a 7½-hour scotophase. These experimental conditions were employed because previous work had shown that over 90% incidence of diapause was induced in the Madison population of the European corn borer when they were grown under a 12–13-hour photophase in a 24-hour photoperiod. Either more or less light induced less diapause. There was no appreciable

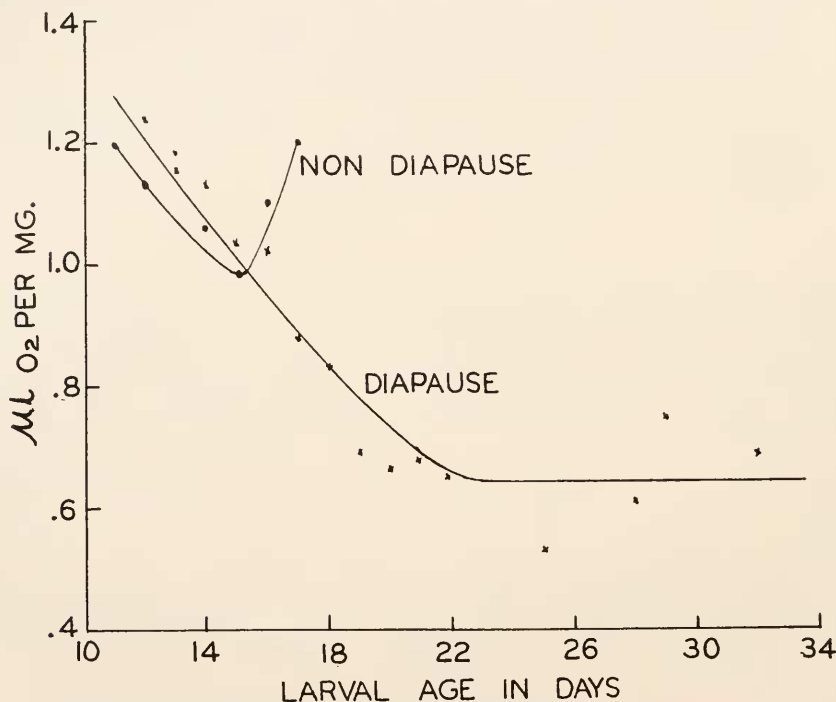


FIGURE 1. Rate of oxygen consumption by diapause and nondiapause larvae of the European corn borer.

diapause in response to a long day, continuous light, or continuous dark (Beck and Hanec, 1960).

Diapause in the individual borer was determined by a negative criterion—failure to pupate. With such a criterion, there had to be an arbitrary point of time selected, after which the borers were considered to be in diapause. This point was reached experimentally when the control borers, reared in the dark, had finished pupating and the pupation curve for the experimental population had leveled off (see Figure 2). Diapause borers were obtained for experimentation by rearing them in a short day for 21 days after eclosion. At this time they were placed in clean vials on wet paper strips. Experiments on breaking diapause were terminated when all the borers in the sample had either pupated or died.

The oxygen consumption of laboratory-reared borers was carried out using standard manometric techniques (Umbreit *et al.*, 1957). Borer larvae were confined in small wire cages in each Warburg flask. This prevented them from crawling into the center well and, because the insect is thigmotactic, also tended to keep them relatively inactive. The oxygen consumption of each larva was measured for one hour in 10-minute increments. The $\mu\text{l. O}_2$ consumed per hour was divided by the live weight of the insect to give $\mu\text{l. O}_2/\text{mg.}/\text{hr.}$

The analyses of variance were calculated by the method of Steel and Torrie (1960) for groups with unequal replication. Duncan's New Multiple Range test

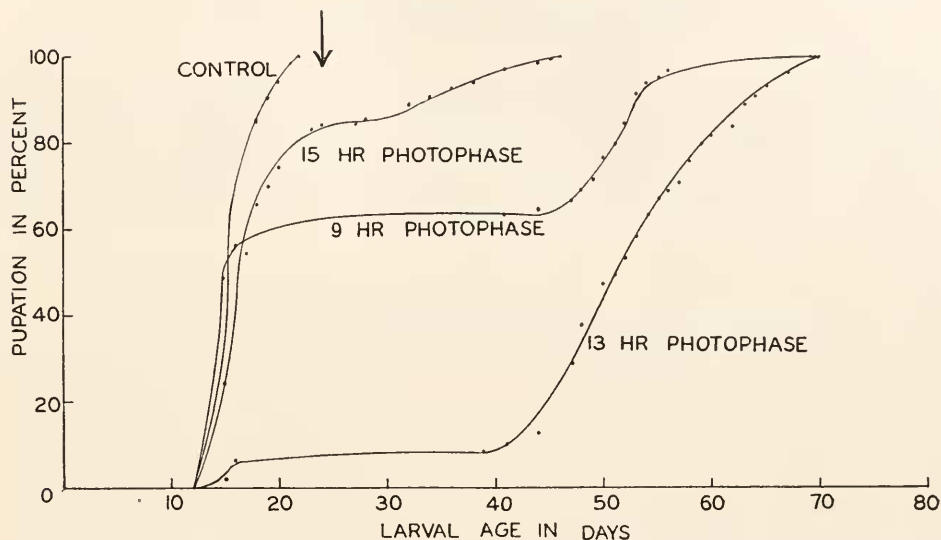


FIGURE 2. Pupation curves for European corn borer populations reared under different photoperiods. The arrow indicates the time at which all diapause larvae were exposed to long-day photoperiods (16.5L/7.5D).

was used with the approximation of Kramer (cited in Steel and Torrie, 1960) for testing means based on unequal replication.

RESULTS AND DISCUSSION

The diapause stage in most insects is characterized by a low level of oxygen consumption (Heller, 1926; Boell, 1935; Schneiderman and Williams, 1953). Beck and Hanec (1960) found that the respiration of borers collected from the field also dropped, but little was known about the length of time necessary to reach this low level of oxygen consumption as the borers entered diapause. The "incipient" diapause reported by Beck and Apple (1961) may have reflected an incomplete suppression of respiration. Once a stable low level of oxygen consumption was reached, the "true" diapause stage would have been attained.

Figure 1 shows that oxygen consumption declined after the moult from the fourth to the fifth instar in both diapause and nondiapause borers. The non-

diapause respiration increased at the pupal moult to form the classical U-shaped curve. Respiration in the diapause larvae continued to drop, stabilizing at from one half to one third the prediapause level. If a stable low level of oxygen consumption is indicative of diapause, then the diapause stage had been reached at this time (22–24 days). If diapause occurred in different intensities after this time, measurement of its intensity would require some method other than oxygen consumption. The average time to pupation when placed in a long day was the criterion used to determine the intensity of diapause in subsequent experiments, the supposition being that the more intense the diapause, the greater the delay in pupation.

The intensity of diapause in larvae of the European corn borer was tested in larvae reared under different photoperiods (Fig. 2). Diapause incidence of greater than 90% was observed among larvae reared under a photoperiod consisting of a 13-hour photophase and an 11-hour scotophase. Intermediate incidence of diapause was obtained under a 15-hour photophase and a 9-hour scotophase and also under a 9-hour photophase and 15-hour scotophase. The 15-hour photophase is on the long-day side of the response maximum, and the 9-hour photophase is on the short-day side of the response peak (Beck, 1962).

TABLE I
The average time to pupation of diapause borers grown under different photophases when transferred to a long day

Photophase (hr./24 hours)	Average time to pupation
9	29.9
13	29.8
15	13.5*

* This mean is significantly different from the other means at the 5% level of probability.

Larvae reared under the 9- and 13-hour photophase treatments reached a maximum incidence of pupation at 15–16 days, and the pupation curve leveled off, while the 15-hour treatment leveled off more slowly (24 days). The dark controls had finished pupating by 22 days. The non-pupators in the three treatments were then considered to be in diapause and were placed in a long-day photoperiod.

When exposed to a long-day photoperiod, the borers reared under the 9- and 13-hour photophase began to pupate in 17–19 days. At from 65 to 70 days of age, all had pupated. The 15-hour photophase treatment, on the other hand, did not result in this pattern. Diapause in these larvae did not appear to be as intense as in the other two groups, and pupation was nearly finished by the time that pupation had started in the other two experimental populations. The average times to pupation, as shown in Table I, were also significantly lower. Different intensities of diapause occurred under different photoperiods, but long-day exposures terminated diapause in borers grown under any of the three photoperiods tested.

There still remained the possibility that longer exposure to a short day would induce a more intense diapause, as shown by de Wilde *et al.* (1959) and Hogan (1962). To test this hypothesis, diapause was induced in a large group of borers with a short-day photoperiod. At 30 days of age, and at subsequent 20-day intervals to 90 days, samples were removed and placed in either continuous dark or

TABLE II

The average time to pupation of diapause larvae sampled at different ages from a population held continuously under a short day

Larval age at transfer (days)	Average time to pupation	
	Cont. dark (days)	Long day (days)
30	78 (2)*	30.7 (23)
50	29 (3)	30.1 (12)
70	17 (3)	21.7 (12)**
90		16.1 (14)**

* Numbers in brackets refer to the number of pupae/sample of 30.

** This mean significantly different from the rest at the 5% level.

a long-day regime, and observed daily for pupation (Table II). Diapause was terminated at any age, and the average time to pupation became shorter with increasing age. The average time to pupation in the 70- and 90-day samples transferred to the long day may have been shortened partly because some of the borers were close to pupation before the long-day treatment was begun.

Diapause in the corn borer does not last indefinitely, even under diapause-inducing conditions. The borers that remained under the diapause-inducing short day started to pupate at about 70 days of age, and by 140 days 36% had pupated and 64% had died. This finding can, perhaps, be explained on the basis that diapause development may proceed at 30° C. Diapause development then proceeds to completion in about one third of the population by 140 days. This would mean that diapause in the borer is much the same as that in *Philosamia cynthia* (Danilevsky, 1949; cited by Lees, 1955). The high end of the temperature range for diapause development corresponds with a large part of the temperature range for morphogenesis. Although diapause development can occur at 30° C., the high rate of mortality and the length of time for pupation would indicate that this is not the optimum temperature for diapause development. It is probable that mortality occurs because the borers have utilized their fat body reserves before diapause development has been completed. An alternative hypothesis is that a diapause of sufficient intensity was not attained under the experimental conditions.

Experiments were set up to determine if a more intense diapause could be induced by varying the temperature and photoperiod to which the insects were exposed. Diapause was induced in two groups of borers, and they were placed in

TABLE III

The average time to pupation of diapause borers stored in continuous dark at 30° C. when transferred to a long day

Larval age at transfer (days)	Days in dark	Average time to pupation in long days (days)
21	0	29.3 (24)*
35	14	29.0 (22)
49	28	24.2 (25)
63	42	28.0 (18)
77	56	27.1 (16)

* Numbers in brackets refer to the number of pupae/sample of 30.

continuous dark at either 30° C. or 5° C. when they were 22 days old. Periodically samples were removed and placed in a long day, and the average time to pupation determined. Tables III and IV show that neither continuous dark nor cold treatment induced a more intense diapause. Diapause was terminated by a long day, and there was no requirement for chilling. The F value from the analysis of variance for the data in Table III was not significant at the 5% level. Table IV shows that the long-day treatments yielded significantly different results. The borers that were exposed to 5° C. for 42 days took less time to pupate than any of the other three groups. But it can be seen from the continuous-dark column (Table IV) that diapause development was completed in very few of the borers during the exposure to 5° C.

In the field, corn borers are not exposed to a constant temperature as in the incubator but to a fluctuating temperature with a low during the night and a high during the day. Beck (1962) found that 96% diapause was induced when borers were grown under a short-day photoperiod and a thermoperiod with the cold during the dark. If the cold came during the light, only 15% diapause was induced.

TABLE IV

The average time to pupation of diapause borers stored in continuous dark at 5° C. when transferred to a long day or continuous dark at 30° C.

Larval age at transfer (days)	Days in dark (5° C.)	Average time to pupation in:	
		Cont. dark (days)	Long day (days)
21	0	— (0)*	27.2 (21)
35	14	52.4 (5)	28.1 (18)
49	28	43.0 (3)	30.7 (9)
63	42	33.0 (1)	19.4 (12)**

* Numbers in brackets refer to the number of pupae/sample of 30.

** This mean is significantly different from the others at the 5% level of probability.

Diapause was induced in a short-day and either a constant temperature (26° C.) or a 21–31° C. symmetrical thermoperiod. The average time required for pupation was compared in the two groups by samples taken at two different times (26 and 50 days). The F values from the split plot analysis of variance were not significantly different at the 5% level for the long-day treatments in Table V. Thus, a thermoperiod imposed on a photoperiodic regime did not induce a more intense diapause.

A diapause incidence of about 45% was induced when borers were reared in continuous dark and a thermoperiod consisting of 16 hours at 32.5° C. and 8 hours at 12° C. with abrupt temperature changes. Borers were reared and held under these conditions for 90 days. Periodically samples were removed and placed in either continuous dark or long day, and observed for pupation. Table VI shows the average time to pupation for these groups. The analysis of variance for the long-day treatments gave a non-significant F value at the 5% level, and it is concluded that a thermoperiod alone does not induce a more intense diapause.

TABLE V

The average time to pupation of borers grown under a short day and either (A) 26° C. constant temperature, or (B) 21–31° C. symmetrical thermoperiod when transferred to a long day or continuous dark

Larval age at transfer (days)	Temperature condition	Average time to pupation	
		Cont. dark (days)	Long day (days)
26	A	108 (1)*	29.8 (25)
26	B	68 (1)	28.2 (23)
50	A	41.5 (5)	28.3 (27)
50	B	59.8 (5)	33.1 (18)

* Numbers in brackets refer to the number of pupae/sample of 30.

The preceding data show that an intense diapause, that is, a diapause that required a prolonged period of chilling for termination, could not be induced. Diapause could always be terminated with a long-day photoperiod. There was no requirement for chilling to break diapause.

Experiments on the response of field-collected borers to a long-day photoperiod were begun at the end of August when pupation was completed in the field population. Samples of borers were collected periodically and placed in either a long day or in continuous dark. Table VII shows the average time to pupation for these groups. The results show that diapause could be broken in the field population by a long day, and that diapause termination did not require chilling. It is evident that the diapause induced in laboratory-reared borers was similar in intensity to that of the field-collected borers.

It is evident from the "continuous dark" column (Table VII) that diapause development had been completed in the majority of borers by December 13. The average time to pupation became shorter after this date, because morphogenesis proceeded slowly at the low temperature in the field. After this date, the average time to pupation in the long day did not differ significantly from that in the dark. Thus, once diapause development had been completed, long-day exposure had no further effect.

The experimental results discussed thus far show that diapause development

TABLE VI

The average time to pupation of diapause borers grown in continuous dark and a thermoperiod of 16 hours 32.5° C. and 8 hours 12° C. when placed in continuous dark or long day

Larval age at transfer (days)	Average time to pupation	
	Cont. dark (days)	Long day (days)
45	68.3 (3/15)*	31.0 (13/15)
60	—	31.7 (18/30)
75	—	26.9 (17/30)
90	21.6 (5/15)	23.1 (14/25)

* Numbers in brackets refer to the number of pupae/number in sample.

can proceed very slowly to completion at 30° C. in continuous dark or under a short day. Long-day treatment greatly accelerates the rate of diapause development. The mortality that occurred when borers were held for a long period of time at 30° C. indicates that borers may need chilling, not to complete diapause development, but rather to decrease the metabolic rate so that there is enough fat body reserve to insure morphogenesis once diapause development has been completed.

Since diapause development can be completed in the corn borer by a long-day photoperiod, a question arises as to the number of days of long-day treatment necessary. To answer this question, diapause was induced in a group of borers by rearing them under a short-day photoperiod for 21 days; they were then placed in a long-day incubator. Periodically samples were removed and placed in continuous dark and the percentage pupation observed at 66 days of age. The experiment was terminated at this age because pupation was finished in the group that

TABLE VII

The average time to pupation of diapause field-collected borers when placed in either a long day or continuous dark

Date collected	Average time to pupation	
	Long day (days)	Cont. dark (days)
Aug. 22	24.7 (25/27)*	71.1 (9/24)
Sept. 6	24.6 (28/30)	93.5 (4/6)
Sept. 20	25.9 (22/30)	124 (1/6)
Oct. 4	27.7 (26/30)	60.0 (2/6)
Oct. 18	24.9 (18/21)	—
Nov. 1	27.0 (24/30)	73.6 (7/21)
Nov. 15	27.4 (27/30)	57.7 (4/21)
Nov. 29**	24.0 (3/30)	29.0 (1/30)
Dec. 13	26.8 (25/36)	26.6 (17/36)
Dec. 27	21.2 (20/30)	26.9 (14/30)
Jan. 10	16.7 (21/30)	20.2 (17/30)
Jan. 24	14.9 (21/30)	16.6 (24/30)
Feb. 7	15.1 (21/30)	17.4 (17/30)
Apr. 24	11.8 (22/27)	11.8 (16/27)

* Numbers in brackets refer to the number of pupae/sample size.

** This collection was heavily infected with a fungus and high mortality occurred.

remained in the long day, and pupation could occur after 70 days among diapause borers held in the dark.

Figure 3 shows that diapause was terminated in 20% of the population by as little as two days of long-day treatment, and with 12–16 days, 100% pupation occurred. It is evident that diapause development induced by the long-day photoperiod took up little of the time (20–30 days) necessary to reach the pupal stage; the remainder of the time was required for morphogenesis.

The terms "diapause" and "diapause development" have been used in this discussion with no attempt to assign these terms to any physiological mechanism, although experimentally these processes have been shown to occur. Wigglesworth (1934) was the first to propose that diapause was linked to the hormones controlling growth and metamorphosis. Subsequently, Williams (1946, 1947, 1948) showed that diapause in the *Cecropia* silkworm was caused by the failure of the neuro-

secretory cells of the brain to secrete the activation hormone. The brain regains its ability to secrete the activation hormone when chilled for 6 weeks at 5° C. The activation hormone stimulates the prothoracic glands to secrete ecdyson, and adult differentiation follows. In this case "diapause" refers to the "metabolic block" preventing secretion of the activation hormone; and "diapause development" to the process taking place at 5° C. that returns the brain to secretory activity. This general scheme has been found to fit the larval diapause in the wheat stem sawfly, *Cephus cinctus* (Church, 1955) and the pupal diapause in the Lime hawk moth, *Mimas tiliae* (Highnam, 1958).

It has been found by Cloutier *et al.* (1962) that the brain of the diapause borer is able to promote adult development in diapause larvae. One of the hy-

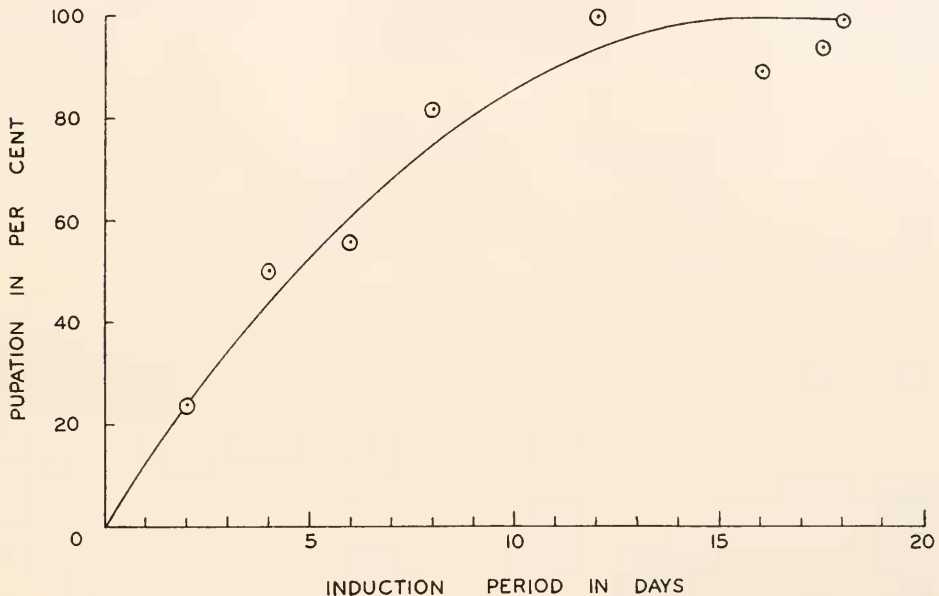


FIGURE 3. Effect of different long-day exposures on the termination of diapause in the European corn borer.

potheses presented to explain this was that the brain contained the activation hormone and that its release was blocked in the intact diapause brain. This situation differs from that found in the giant silkworm, *Hyalophora cecropia*; Williams (1948) found that up to 8 diapause brains could not promote adult development in diapause pupae. Thus, it appears that diapause development in the European corn borer facilitates the release of the product, and not its production.

The sequence of events that appears to take place before pupation can occur is: (1) removal of the block preventing release of neurosecretory products; (2) secretion of the activation hormone in sufficient titer to stimulate the prothoracic glands; and (3) production and secretion of ecdyson in sufficient titer to promote adult differentiation. It is well substantiated that once the prothoracic glands have been stimulated sufficiently by the activation hormone, adult differentiation can proceed

TABLE VIII

The per cent pupation after the re-induction of diapause

Days in long day	Pupation when transferred to	
	Short day %	Cont. dark %
0	0	—
7	0	48
9	33	53
11	67	79
Continuously in long day 100		

without further brain activity (Williams, 1946). This is also true for the corn borer. Of 24 pupae that had their brains removed immediately after pupation, the 22 survivors underwent adult differentiation and emerged as moths. This showed that sufficient activation hormone had been liberated prior to the pupal moult to stimulate adult differentiation, and that the prothoracic glands could be effective in the absence of the brain. If, in terminating diapause with a photoperiodic cycle, secretion of the activation hormone takes appreciable time to reach a level high enough to stimulate the prothoracic glands, there might be some point after diapause development has been completed where the block to secretion might be re-established by a short day. Thus, it could be possible to re-induce diapause by physiologically "removing" the brain.

To test this hypothesis, diapause borers were placed in a long day to induce diapause development. Periodically duplicate samples were removed and placed in either continuous dark or short day. After 45 days from the beginning of the long-day treatment, the percentage pupation was calculated in all groups. Table VIII shows that diapause could be re-induced in all those borers that had only 7 days of long-day treatment and a small percentage could be made to re-enter diapause after 9 and 11 days of long-day treatment. This experiment does not take into account the time necessary to establish the block to neurosecretion, but at least 7 days is not time enough to stimulate the prothoracic glands. But from those samples placed in continuous dark, it can be seen that 7 days of long-day treatment was enough to terminate diapause in 48% of the population. It would thus appear that secretion of the activation hormone is a part of morphogenesis and not diapause development, and hence is not effected by photoperiod.

In the previous experiment the borers were transferred to a short day and remained there until the end of the experiment. As diapause could be reinstated in all after 7 days of long-day treatment, this time was used to determine how many

TABLE IX

The per cent pupation at 45 days when different lengths of time for diapause re-induction were used

Days in long day	Days in short day	Per cent pupation
7	0	46
7	4	40
7	8	20
7	12	6

days of short treatment were necessary to re-induce diapause. This experiment was set up in the same manner as the previous one except that the borers were removed from the short-day incubator at various times and placed in continuous dark. The per cent pupation was calculated in all groups 45 days after the transfer to the long day (66 days of age).

Table IX shows that at least 12 days of short-day treatment are necessary to reduce the per cent pupation from 46% to 6%. It appears, then, that considerable time is required to re-establish the block to neurosecretion. The short length of time necessary for diapause development in a long day compared with the length of time necessary to induce diapause is quite in agreement with what is known about the original induction of diapause (Beck and Hanec, 1960).

Borers collected from the field on October 28 and stored at 5° C. in the dark for 53 days were subjected to different photoperiods. The average times to pupation in Table X were not significantly different at the 5% level. Thus, photoperiod

TABLE X

Average time to pupation of field-collected borers under different photoperiods

Photoperiodic condition	Average time to pupation
Continuous dark	18.3
Continuous light	15.4
Long day	15.7
Short day	13.9

had no effect on these borers. These findings imply that diapause development had been completed, and that morphogenic development had advanced beyond the point where it could be arrested.

SUMMARY

1. The European corn borer, *Ostrinia nubilalis*, has a facultative diapause in the last larval instar. Diapause induced in the laboratory by a short-day photoperiod is identical in its intensity to that occurring in the field.

2. Diapause development occurs at 30° C. under various photoperiodic conditions but is greatly accelerated by a long day.

3. Completion of diapause development does not require a period of chilling.

4. Diapause development is a process that removes a block to secretion of the activation hormone but does not include secretion or any of the morphogenic events that follow.

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